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6. AUTHOR(S)
Dr Benjamin Rusak

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
Dept of Psychology
Dalhousie University
Halifax, Nova Scotia
CANADA B3H 4J1

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13. ABSTRACT *(Maximum 200 words)*

This program of research identified a number of novel roles for peptides and neurotransmitters found in the suprachiasmatic nuclei (SCN), in the regulation of SCN neuronal activity and behavioral circadian rhythms. Major findings included the observations of changes in SCN cell firing rates and behavioral phase shifts induced by gastrin releasing peptide (GRP) when applied to SCN cells. GRP was demonstrated not to act primarily in concert with other peptides in a manner proposed by an earlier publication. Similar combinations of neurophysiological and behavioral studies have been completed using another peptide, substance P, and are partially completed using VIP. Other studies have investigated the neurophysiological role of metabotropic glutamate receptors, and the behavioral effects of their activation or blockade. Neurophysiological studies investigated the effects of melatonin and of serotonin on photic responses of SCN cells and evaluated putative antagonists for these effects. Antagonists that acted selectivel on the melatonin and serotonin receptors in the sCN were identified. Additional studies examining the neurophysiological effects of opiatergic drugs, histamine and redox agents were also completed.

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Title: Neurophysiological analysis of circadian rhythm entrainment

Contract #: F49620-93-1-0089.

Principal Investigator: Benjamin Rusak

Institution: Dalhousie University

Abstract

This program of research identified a number of novel roles for peptides and neurotransmitters found in the suprachiasmatic nuclei (SCN) in the regulation of SCN neuronal activity and behavioral circadian rhythms. Major findings included the observations of changes in SCN cell firing rates and behavioral phase shifts induced by gastrin releasing peptide (GRP) when applied to SCN cells. GRP was demonstrated not to act primarily in concert with other peptides in a manner proposed by an earlier publication. Similar combinations of neurophysiological and behavioral studies have been completed using another peptide, substance P, and are partially completed using VIP. Other studies have investigated the neurophysiological role of metabotropic glutamate receptors, and the behavioral effects of their activation or blockade. Neurophysiological studies investigated the effects of melatonin and of serotonin on photic responses of SCN cells and evaluated putative antagonists for these effects. Antagonists that acted selectively on the melatonin and serotonin receptors in the SCN were identified. Additional studies examining the neurophysiological effects of opiatergic drugs, histamine and redox agents were also completed.

Work accomplished during the grant period

1. Behavioral and neurophysiological effects of light

In collaboration with Dr. J. Meijer, we completed a study designed to investigate the dynamics of how single SCN cells respond and habituate to light exposures, and to compare these responses to those of the whole circadian system. We compared the firing-rate responses of single SCN neurons in hamsters to continuous 15 min light presentations and to a series of eight 1 min pulses separated by 1 min periods of darkness. The neurophysiological responses were then compared to the behavioral phase-shifting responses of hamsters exposed to trains of one, two, four or eight 1 min light pulses.

The neurophysiological results indicated that responses to light pulses at the end of the train were robust and similar in amplitude to those at the beginning, while there was a gradual reduction of firing rate for most cells during the latter portion of a continuous 15 min light exposure. Behavioral results indicated that additional light pulses added additional phase-shifting effects, but that later pulses in the train added progressively less phase shift. Thus, neurophysiological responses (assessed as the total number of spikes counted during a train of light pulses) increased linearly with added light pulses, while phase-shifting effects increased at most logarithmically with added pulses. Firing rate increases in SCN cells may faithfully reflect photic input to the SCN, but the pacemaker appears to introduce non-linearities in the functional response to this input. These results have been published [1].

2. Neurophysiological effects of melatonin

The pineal hormone melatonin plays an important role in the regulation of circadian rhythms in vertebrates other than mammals, but its role in mammals is less clear. We have been studying the effects of melatonin on photic responses of SCN and IGL cells, and for comparative purposes, also on activity of cells in the hippocampus and cortex. In addition, we began to study the effects of naphthalenic melatonin analogs manufactured by Servier (Paris), some of which are supposed to be potent melatonin receptor agonists and antagonists. After we had completed our initial studies using these analogs in 1994, Servier proposed a contract to conduct further studies on these drugs with their financial support. We have published several abstracts and reports based on our initial studies of melatonin and its naphthalenic analogs [5,11,16,19,28,30,33].

Our first studies concerned the role of daily pineal melatonin rhythms in the regulation of rhythmic sensitivity to melatonin and firing-rate rhythms in the hamster SCN slice preparation. We examined the effects of both acute exposure to constant light for several days (to suppress melatonin secretion) and of pinealectomy a week before slice preparation on the rhythm of SCN cell sensitivity to melatonin and on SCN cell firing-rate rhythms. We found that either treatment flattened the rhythm of melatonin sensitivity, suggesting that the presence of melatonin may modulate the availability of its own receptor [2,3], a conclusion that has been confirmed by recent receptor-binding studies, although not all results are consistent with it. Our results also indicated that pinealectomy reduced the amplitude of the firing-rate rhythm in the slice [3]. This result suggests a mechanism whereby pinealectomy or loss of normal melatonin levels (e.g., with aging in humans) may interact with other stressors on the circadian clock mechanism to produce severe rhythm disruptions.

We subsequently studied the effects of melatonin as a modulator of photic responses in both the IGL and the SCN of hamsters. These studies involved single-unit recordings in urethane-anesthetized animals. We found that melatonin reduced the photic responses of both IGL and SCN cells in a dose-dependent manner and had a generally inhibitory effect on them, although it activated a small proportion of cells, as it did in the slice preparation [5,11]. Unlike the effects of serotonin (5-HT), the effects of melatonin could not be attenuated by the 5-HT receptor antagonist metergoline, suggesting that the

effects are mediated by a melatonin receptor and not by the interaction of melatonin with serotonin receptors.

In our initial studies using Servier's naphthalenic analogs of melatonin, we demonstrated that S-20098 acts as a potent agonist with suppressive effects on photic responses of both SCN and IGL cells. These effects were similar to those of melatonin but were somewhat more sustained, at least when the agonist was injected intraperitoneally (IP). The putative melatonin antagonist S-20928 actually acted as a partial agonist when injected IP at higher doses, but at low doses did not alter photic responses by more than 10-20%. Co-application of S-20928 (at doses that lacked agonist activity) along with melatonin or S-20098 potently attenuated the agonists' effects on SCN cell photic responses. This drug appears, therefore, to act as a specific melatonin antagonist at low doses, despite having mixed properties at higher doses [11]. These results are illustrated in Fig. 1, below.

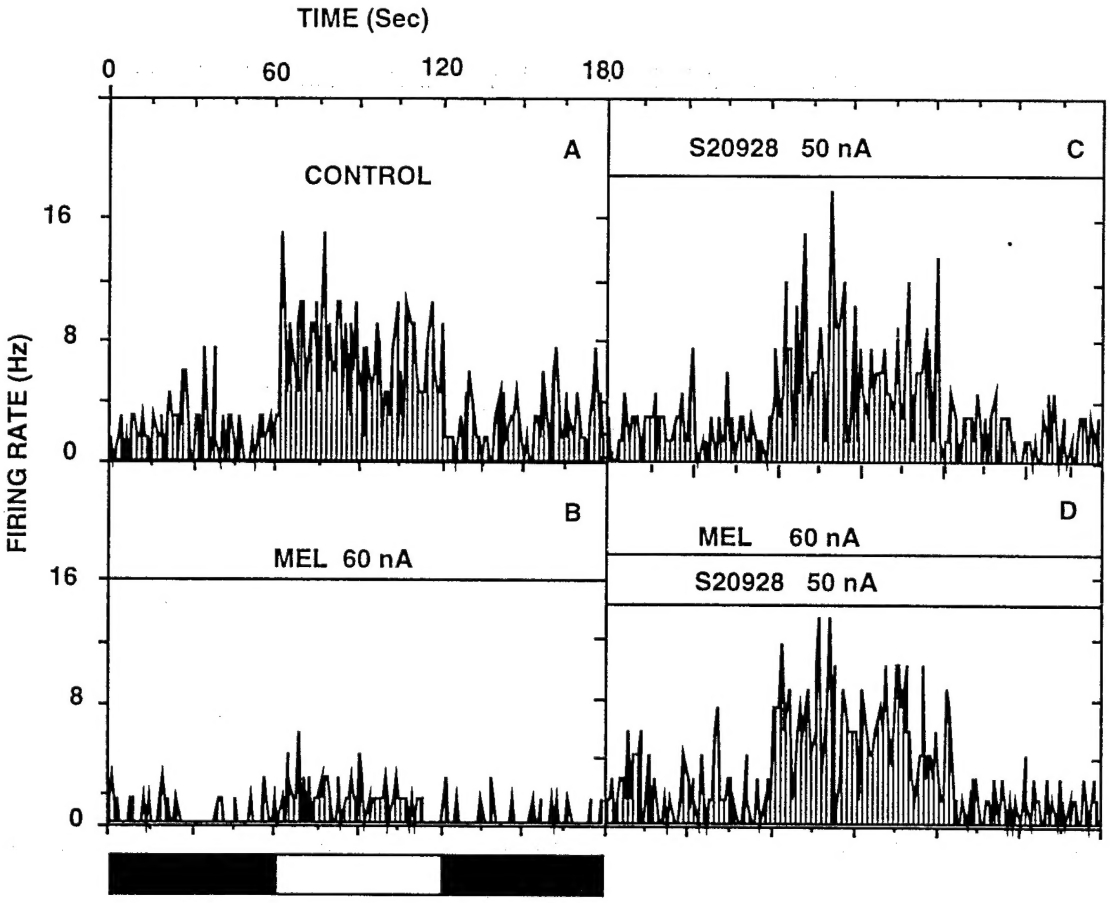

Fig.1. Effects of melatonin and a putative antagonist on photic responses of a hamster SCN cell. A: Response of the cell to retinal illumination delivered in the pattern shown below the figure. B: Suppression by iontophoresed melatonin of both spontaneous and light-evoked activity. C.: Slight effect of the putative antagonist given IP on its own. D: Blockade of melatonin effects by IP injection of the antagonist. (from Ying et al. [11])

Because of the poor solubility of these drugs in physiological solutions, they could not be used readily in microiontophoretic or micropressure ejection studies in vivo in order to specify their locus of action. We were, however, able to use direct iontophoresis of S-

20098 to study a subgroup of SCN and IGL cells, and to confirm that the agonist acts directly on SCN and IGL cells. We are now studying these drugs in a slice preparation in which micropressure ejection studies are more feasible, and our preliminary results support the interpretation that they act on SCN cells directly (G. Scott, unpublished observations; [33]).

3. Neurophysiological effects of 5-HT

There has been increasing interest in the role of serotonin (5-HT) in the mammalian circadian system for a variety of reasons: there is a prominent serotonergic projection from the raphe nuclei to the SCN; 5-HT or its agonists can phase shift circadian rhythms in a slice or in vivo; and damage to the serotonergic system modifies photic entrainment. We studied the effects of selective 5-HT receptor agonists and antagonists on photic responses in the circadian system of hamsters. 5-HT or its agonists 8-OH-DPAT [(±)-8-hydroxy-2-(D1-N-propylamino) tetralin hydrobromide] and 5-CT (5-carboxamidotryptamine) caused dose-dependent suppressions of spontaneous firing activity and photic responses of both SCN and IGL cells. The effects of 5-HT agonists were blocked by co-application of metergoline, a relatively non-selective 5-HT antagonist, and pindobind-5-HT1A, which is effective on 5-HT1A receptors (but not exclusively so). We also found that SDZ 216,525 (a reportedly selective 5-HT1A antagonist from Sandoz) could attenuate the effects of 8-OH-DPAT, while other putative 5-HT antagonists were weak (propranolol and NAN-190) or ineffective (ketanserin). Our results suggested that 5-HT was acting via a receptor with properties similar to those of 5-HT1A receptors, but with some significant differences [5,6,7]. Our findings could not, however, discriminate effects on 5-HT1A and the newly identified 5-HT7 receptors, since a number of the drugs we used have similar affinities for these subtypes.

To determine whether the receptors mediating 5-HT effects on SCN and IGL cells in hamsters are similar to the 5-HT7 or 5-HT1A receptors, it will be useful to have antagonists that are selective for the 5-HT7 receptor; however, these are not yet available. Instead, we have begun studies using combinations of agonists and antagonists that have relatively higher affinity for either 5-HT1A or 5-HT7 receptors to try to characterize the receptor subtypes involved. Briefly, we have preliminary data from a few cells that the effects of a 5-HT agonist with high affinity for both receptors (8-OH-DPAT) are blocked by an antagonist which has a high affinity for the 5-HT7 receptor (ritanserin), but they are not affected by antagonists that are highly selective for 5-HT1A receptors (WAY100,135 and WAY100,635). These results point to mediation of 5-HT agonist effects via a receptor that more closely resembles the 5-HT7 than the 5-HT1A receptor. A sample of these results is illustrated in Fig. 2 .

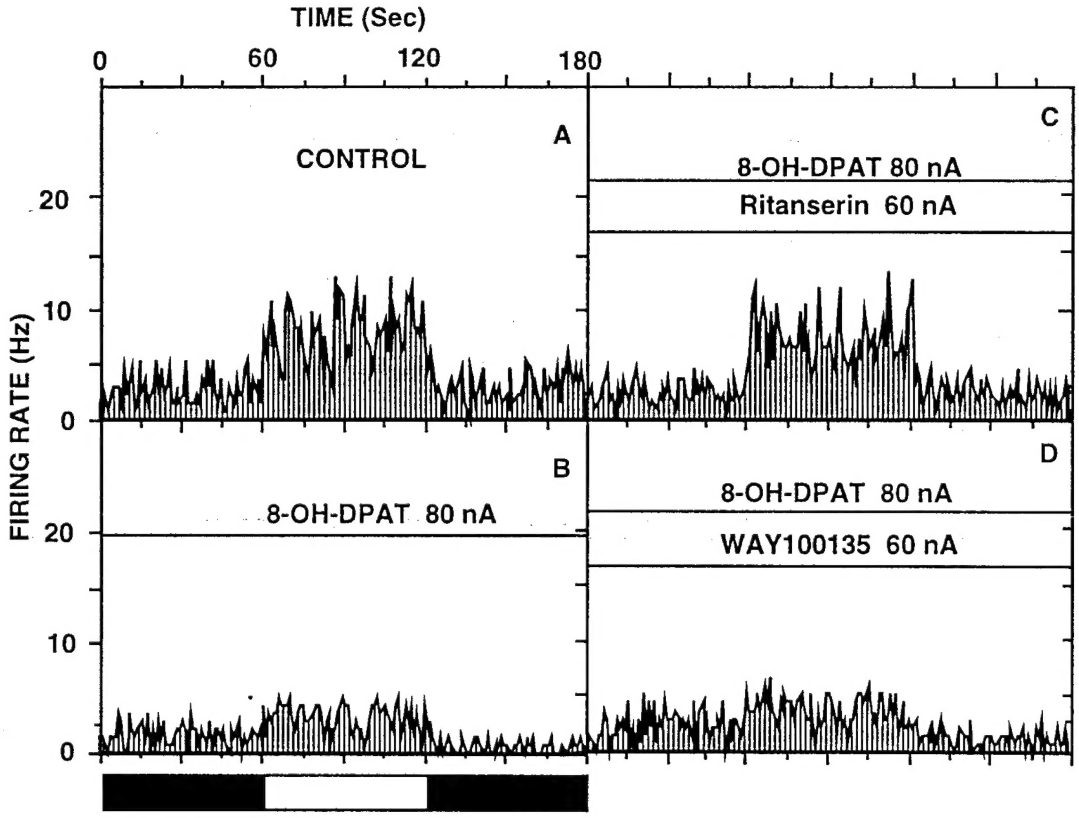

Fig. 2. Effects of the 5-HT agonist 8-OH-DPAT and putative 5-HT antagonists on hamster SCN cell firing-rate responses to light. A: Response of an SCN cell to retinal illumination. B: Suppression of spontaneous and light-evoked activity by iontophoresis of 8-OH-DPAT. C: Antagonism of 8-OH-DPAT effects by co-application of the antagonist ritanserin. D: Failure of the selective 5-HT1A antagonist WAY100,135 to antagonize effects of 8-OH-DPAT. (From Ying and Rusak, unpublished observations, 1995)

4. Behavioral and neurophysiological effects of GRP, VIP and PHI

Gastrin-releasing peptide (GRP), vasoactive intestinal polypeptide (VIP) and peptide histidine isoleucine (PHI) are peptides found in the ventral portion of the SCN, with VIP and PHI being colocalized products of the breakdown of a prepropeptide. VIP-containing cells are innervated by retinal ganglion cells and by 5-HT containing raphe afferents. One report has described a degree of colocalization of GRP with the other two peptides in rat SCN cells. In our anatomical studies, however, the distributions of VIP and GRP immunoreactive cells in rat and hamster SCN have very little overlap, and these cells also have quite different efferent projection patterns, suggesting minimal cellular colocalization (Piggins et al., unpublished observations). We were interested in a report that these three peptides interact in a unique facilitatory fashion, with each peptide having minimal neurophysiological and behavioral phase-shifting effects on its own, but with strong phase-shifting and neurophysiological effects emerging when the three are co-applied. The model proposed to explain these results was potentially an important one because it provided an explanation for why the SCN (and numerous other structures) might contain multiple peptides, and also provided novel insights into the physiology of photic entrainment in mammals.

We assessed the claim that GRP applied alone by bath application has little influence on SCN cell firing rates in a slice preparation. Using micropressure ejections of

GRP1-27, GRP18-27, and bombesin, we established that each of these ligands for the bombesin-preferring receptor has potent neurophysiological effects on approximately 50% of SCN cells [4]. In a subsequent study, we used microiontophoresis to apply GRP analogs and confirmed that electrophoretically mobile analogs that affect the bombesin-preferring receptor are potent modulators of SCN neuronal activity. Responsiveness to GRP18-27 increased from low levels in the subjective day to higher levels during the subjective night. In addition, we showed that these effects can be blocked by application of specific bombesin receptor antagonists [8].

The discrepancy between our findings and those of Albers et al. (1989), did not result from the use of different drug application techniques, since independent studies have now confirmed that bath application of GRP activates about the same proportion of SCN cells as we observed using micropressure or microiontophoretic ejections. Having established that GRP alone can affect SCN neuronal activity through specific receptors and with a daily rhythm of effectiveness, we pursued the question of whether these neuronal changes translate into functional consequences for circadian rhythm phase. In a series of studies involving a wide variety of control conditions and large numbers of animals, we investigated whether application of GRP1-27, PHI or VIP alone to the SCN in vivo can phase shift hamster circadian rhythms, and whether the co-application of these three peptides strongly potentiates their effects, as proposed by Albers et al.

Our first observation was that GRP1-27 caused both delay and advance phase shifts in hamsters housed in constant dim red illumination, during the subjective night when light has similar effects, but not during the subjective day when light is ineffective. This result contrasted with the earlier claim that GRP1-27 caused only delay shifts of small amplitude and no advance shifts in animals housed in constant bright illumination. We assessed whether this difference resulted from the different background illumination conditions used by repeating these studies with animals housed in total darkness and in constant bright illumination. Our findings replicated our earlier result showing substantial phase shifts to GRP with temporal sensitivity that resembled that for light pulses, and it was clear that background illumination condition had no effect on GRP-induced phase shifts [10]. Other control studies established that the phase-shifting effects we observed are dose-related, but independent of injection volume at a fixed drug dose, thereby ruling out another potential cause of the reported differences.

In a final series of studies, we established that VIP and PHI can also cause phase shifts when injected alone into the SCN during the subjective night. Neither was as effective as GRP in causing delay shifts, but VIP was slightly more potent than GRP in causing advance shifts. The shifts produced by combining equimolar doses of the three peptides were on average smaller than the sum of the effects of the component doses given alone [10]. Rather than being mutually potentiating, as had been claimed, or at least showing additive effects, the combination of drugs had sub-additive phase-shifting effects. This observation may reflect the fact that VIP and PHI compete for the same receptor, and a single peptide dose may have been sufficient to largely saturate these receptors. These results contradict the model that proposes that these peptides are largely ineffective on their own and require co-release to exert significant functional effects. Our recent anatomical evidence (Piggins et al., unpublished observations) that the distribution of GRP-containing neurons has very little overlap with the distribution of VIP-containing neurons in the hamster SCN also makes the model inherently less plausible. Our results also establish that GRP and related peptides in the SCN function on their own as potent phase-shifting agents, with phase-sensitivity that is similar to that for light pulses. The results of this series of six studies have been published in a single comprehensive paper in the *Journal of Neuroscience* [10].

5. Effects of Substance P

Substance P (SP) has been suggested as a peptide that may play a role in photic entrainment. SP is found in fibers and cell bodies in the rat and hamster SCN and has been

identified in some retinal ganglion cells projecting to the rat SCN. SP application has been reported to phase shift firing-rate rhythms in the slice with a light-like phase response curve and to alter SCN cell firing rates in rats. We examined the effects of SP applied iontophoretically to hamster SCN cells in a slice preparation.

We found that SP increased firing rates of ~30% of SCN cells tested, and decreased firing rates in less than 10% of cells. Although SCN cells showed a spontaneous firing-rate rhythm in the slice, the effects of SP application did not appear to vary with circadian phase. SP co-application potentiated or added to the activation evoked by application of excitatory amino acid (EAA) agonists in about half the cells tested [9]. These results confirm that SP can affect SCN neuronal activity and can interact with transmitters suspected to participate in photic entrainment. Subsequent behavioral studies in hamsters have failed to show consistent evidence that SP application to the SCN can cause significant rhythm phase shifts in vivo [32]. It remains unclear whether SP plays a significant role in photic entrainment processes in hamsters. The localization of cells expressing the SP receptor in the periphery of the SCN, and the lack of evidence for SP-containing retinal ganglion cells innervating the hamster SCN (Piggins et al., unpublished observations) also do not support the idea that SP plays a central role on its own in photic entrainment, although the possibility that it interacts with other transmitters to affect entrainment remains to be assessed.

6. Effects of glutamate receptor agonists and antagonists

There is strong reason to suspect that glutamate, the dominant excitatory transmitter in the hypothalamus, is also the major transmitter conveying photic information to the SCN via the RHT. It remains uncertain, however, which of the many cellular effects of glutamate contribute to its light-regulated modulation of SCN activity and of circadian rhythm phase. Most research on this issue has focused on the several classes of ionotropic glutamate receptors, characterized by their affinity for N-methyl-D-aspartate (NMDA), α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA), and kainate. But in addition to exerting effects on ion channel activity via ionotropic receptors, glutamate acts via G-protein coupled, metabotropic receptors (mGluR's) to affect cell metabolism; e.g., by stimulating the hydrolysis of phosphatidylinositol diphosphate into second messenger products (diacylglycerol and IP3), or inhibiting adenylate cyclase activity.

At least two classes of metabotropic receptor subtypes (mGluR1 and mGluR5) have been identified in the SCN, so we undertook the first neurophysiological and behavioral studies of the roles of metabotropic receptors in the SCN. In a neurophysiological study of hamster SCN cells in a slice preparation, we examined whether an agonist specific for metabotropic receptors (1S,3R-ACPD) affected SCN cell firing rates and whether a phenylglycine derivative that acts as a specific metabotropic antagonist (RS-α-MCPG) could block the effects of 1S,3R-ACPD and of the ionotropic ligand NMDA. In a paper recently published in *Neuroscience* [12], we report that 1S,3R-ACPD can activate 65% of SCN cells, and that these effects are selectively reduced by co-application of RS-α-MCPG. All cells that were activated by 1S,3R-ACPD were also activated by NMDA, but the metabotropic antagonist RS-α-MCPG did not alter NMDA-induced activations.

We observed a strong regional difference in sensitivity to 1S,3R-ACPD within the SCN. Cells in the ventral SCN responded briskly to brief applications of 1S,3R-ACPD and reached peak firing rates as high as those induced by NMDA. There was a prolonged recovery toward baseline firing rates, even after very short periods of drug application. By contrast, cells in the dorsal SCN showed sluggish responses to 1S,3R-ACPD which did not reach high firing rates and which recovered to baseline immediately after cessation of drug iontophoresis. Unlike NMDA, which was equally effective in all parts of the SCN, 1S,3R-ACPD was most effective in the ventral SCN, where the densest retinal innervation is found. According to one study, the ventral SCN is also the only region of the nucleus containing mGluR1 receptors, at least in adult rats, while another described both mGluR1 and mGluR5 receptors as being restricted largely to the ventrolateral SCN.

This evidence for effects of 1S,3R-ACPD on SCN cell firing rates led us to assess the phase-shifting effects of microinjections of 1S,3R-ACPD into the SCN in intact hamsters freerunning in constant darkness (Scott et al., unpublished observations). Doses of 1S,3R-ACPD ranging from 0.2 mM up to 5 mM failed to shift hamster activity rhythms when injected at circadian times (CT) 6, 13 or 18 (with CT 12 defined as activity onset). We then assessed the effects of RS-α-MCPG (2 mM) on both phase delays and advances induced by brief light pulses. We used relatively dim lights (10-15 lux), since in previous studies NMDA antagonists reduced the amplitude of phase shifts induced by light at similar intensities. We compared the effects of RS-α-MCPG to those of dizocilpine (MK-801; 1 mM) injected before light exposure. Either treatment reduced light-induced phase shifts by ~50%. A manuscript describing these findings is being prepared for submission.

The attenuation of light-induced phase shifts by RS-α-MCPG suggests that mGluR's contribute to the effects of light, along with ionotropic receptors. But the failure of 1S,3R-ACPD to elicit significant phase shifts on its own suggests that activation of mGluR's alone is insufficient to mimic the effects of light. One possibility is that both ionotropic and metabotropic receptors must be co-activated to get maximal effects.

We have also studied the neurophysiological effects of manipulation of the redox state of a site on the NMDA receptor channel complex. This site may be represented by a pair of cysteine residues on the extracellular surface of the channel that form a disulphide bond. There is evidence from other systems that NMDA currents can be influenced by the redox state of this site. Reducing this site with agents such as dithiothreitol (DTT) increases the current flow through the channel, whereas oxidizing the redox site by using 5,5-dithio-bis-2-nitrobenzoic acid (DTNB) decreases the current flow.

The effects of iontophoretic application of DTNB and DTT on NMDA-evoked activity in the SCN were studied in a hamster brain slice preparation. Short applications of NMDA (5-20 s; 10-50 nA) at regular 2 min intervals elicited potent increases in spontaneous neuronal activity, as previously reported. Co-application of DTNB (2 mM or 10 mM; 10-100 nA; 10-300 s duration) had no effect on NMDA-evoked activity in the large majority of cells activated in such a manner, but spontaneous activity was attenuated in most SCN cells. Co-application of DTT (10 mM; 10-100 nA; 10-60 s duration), however, appeared to potentiate both NMDA-evoked responses and spontaneous activity. In addition, prolonged application of NMDA (>60 s) elicited a maintained increase in SCN cell activity which was attenuated by co-application of DTNB in some cells, but these results have been variable (Scott and Moran, unpublished observations). These preliminary data suggest that DTNB has different effects depending on the duration of NMDA receptor mediated activity.

7. Effects of histamine

Histamine originates in neurons in the tuberomammillary hypothalamus, and is found in fibers in the SCN. Previous studies found that histamine elicits both excitations and inhibitions of spontaneous neuronal activity in the rat suprachiasmatic nucleus, effects attributed to activation of H1 and H2 receptors, respectively. In addition, there is evidence that intracerebroventricular injections of histamine cause phase delays (and possibly phase advances) in free-running locomotor activity rhythms of rats, and that histamine phase shifts the rhythm in neuronal firing rate in the SCN in vitro. These findings suggest that histamine may play a role in modulating mammalian circadian rhythm entrainment. We have investigated this issue using neurophysiological and behavioral studies in hamsters.

Iontophoretic application of histamine to hamster SCN neurons in a slice preparation elicited a variety of responses. Histamine either increased or decreased spontaneous activity of SCN cells, possibly through actions at different histamine receptor subtypes. Application of the selective H2 receptor agonist dimaprit also caused current-dependent increases in hamster SCN cell activity. Applications of antagonists selective for H1 and H2 receptors (chlorpheniramine and cimetidine, respectively) failed, however, to block either activation or suppression of cell activity by histamine. One concern, however,

is that at higher ejection currents these antagonists have apparent membrane stabilizing effects that might interfere with assessment of their antagonist properties. Bearing this concern in mind, these results may indicate that histamine can act via a mechanism that does not involve binding to classical histamine receptors.

A precendent exists in previous studies of hippocampal neurons, which found that histamine can exert a modulatory effect on the NMDA receptor complex, rather than acting on a classical histamine receptor. Consistent with this hypothesis, we found that histamine, at ejection currents that have no effect on spontaneous activity, potentiated activations evoked by NMDA application on SCN cells. Histamine may have several effects on SCN cells, some involving binding to histamine-selective receptors, and others involving modulation of glutamate-sensitive NMDA receptors [29].

In behavioral studies, we examined the effects on freerunning activity rhythms of injecting histamine or dimaprit directly into the SCN of hamsters. Injection of histamine (0.5-1 mM) into the SCN did not cause significant phase shifts at CT 6, 13 or 18. Injection of dimaprit (1 mM), however, elicited small, but significant, phase delays at CT 18-22 (22 $\pm$ 3 min). The lack of substantial histamine effects on hamster rhythms may reflect the fact that it acts on a variety of receptors in ways that may offset any phase-shifting effects mediated through one receptor subtype. An abstract describing these initial studies of histamine is in press [29] and a full manuscript has been submitted to *Neuroscience*.

8. Effects of opioid drugs

Two peptides have been identified in the projection from the IGL to the SCN, NPY and enkephalin. The NPY projection has been studied with respect to its neurophysiological effects in the SCN and its functional effects on rhythms. Much less is known about the enkephalin-containing component of this projection, but opioid receptors which could be targets for this projection have been identified in the SCN. With the support of the AFOSR, we have collaborated with Dr. Robert Mason and David Cutler (a graduate of this laboratory) of the University of Nottingham to begin to study the effects of opioids on the circadian system.

Initial neurophysiological studies in a slice preparation employed a bath perfusion method to assess whether a range of opioid agonists (leu-enkephalin, morphine and dynorphin) acting at different opioid receptors can alter SCN neurophysiology. The results indicated that these opioid agonists had little or no effect on SCN cell firing rates on their own. Nor did the co-application of opioid agonists significantly alter the activations induced by NMDA. Despite these unpromising results, one set of observations suggest that endogenous opioids may play a role in the regulation of SCN activity. Following prolonged (e.g., 5-20 min) opioid agonist exposure, which had little or no effect on baseline firing rate, there was a dramatic rebound ("withdrawal") increase in firing rate when either the agonist perfusion was stopped or the opioid receptor antagonist naloxone was co-applied. Naloxone alone, in the absence of opioid agonist treatments, had no effect, regardless of whether it was perfused or applied by micro-ejection. These observations imply a function for enkephalin from the IGL, but the nature of this function is unclear.

Publications Supported by AFOSR

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